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Supplemental Data

C. elegans Major Fats Are Stored in Vesicles Distinct from Lysosome-Related Organelles

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Supplemental Materials:

Figure S1

Figure S2

Supplemental Experimental Procedures

Figure S1. Lysosome-Related Organelles and Neutral Lipid Vesicles Are Mostly Non-Overlapping Compartments. (A) Neutral lipid droplets stained post fixation with LipidTOX red do not co-localize with C1-C12 BODIPY positive organelles. (B) C1-C12 BODIPY labeled-fatty acids signal co-localizes with the lysosome-specific fluorescent dye LysoTracker red.

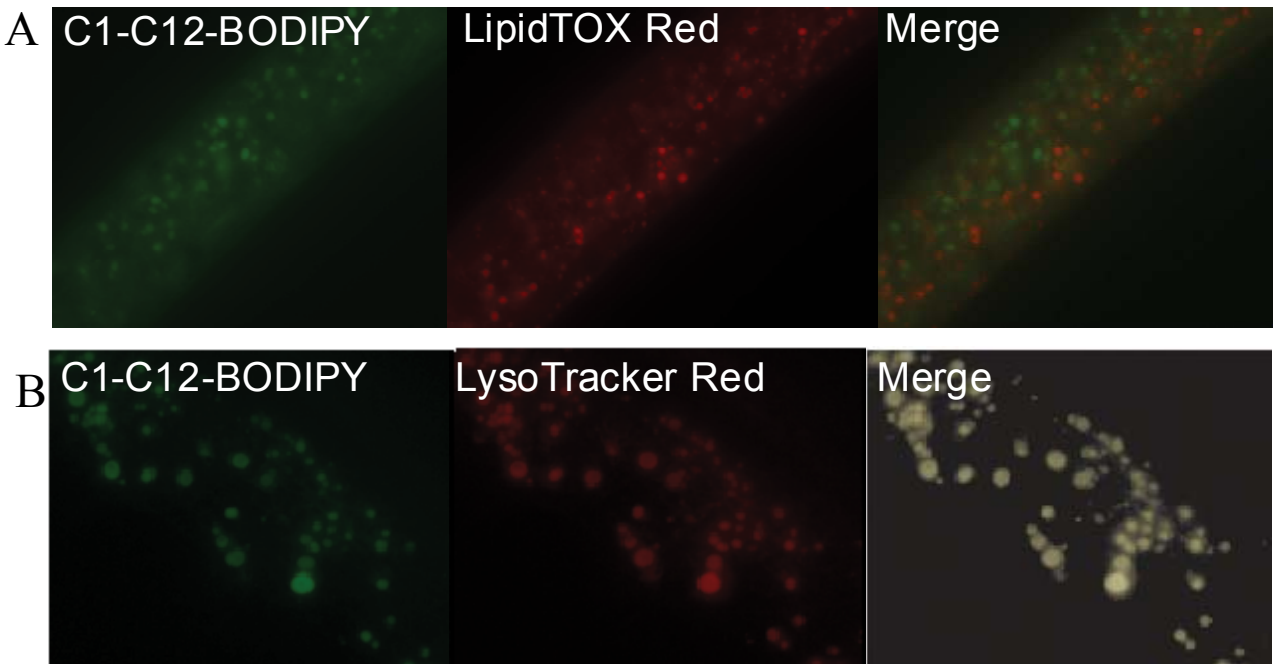
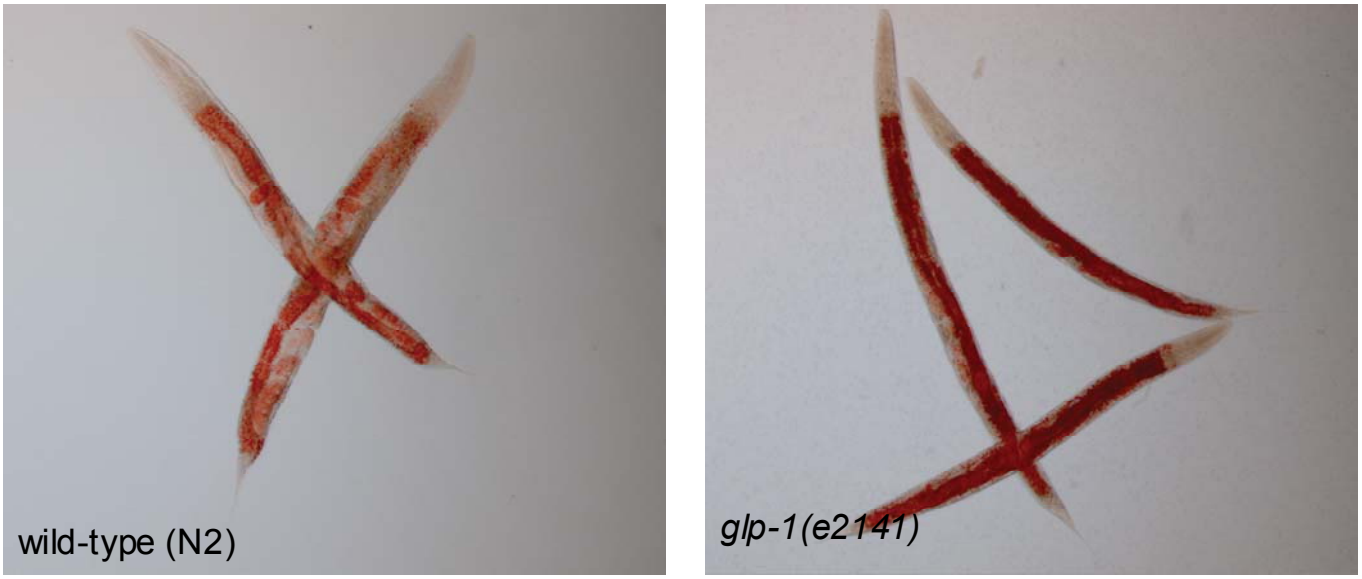


Figure S2. *glp-1* animals accumulate more fat that wild-type animals. Oil-Red-O representative images of wild-type and *glp-1(e2134)* shifted as L4s to 25°C for 24h.



Supplemental Experimental Procedures

Nile red and BODIPY-labeled fatty acid *staining*

Nile red and BODIPY-labeled fatty acid analyses were conducted as previously described(Mak et al., 2006). Briefly, 0.2µg of the fluorescent fatty acid analogue C1-BODIPY 500/510-C12 (Invitrogen) or 0.5µg of Nile red (Invitrogen) were added in 100µl of S-basal to 6cm NGM plates containing 12 mL of media on top of the *E. coli* OP50 lawn (final concentrations 16 ng/mL and 41 ng/mL, respectively). Wild-type or mutant *C. elegans* were seeded by a 2 hour synchronous egg lay or as L1 following overnight hatching and synchronization at 20 ºC in minimal media. Imaging and quantification was conducted after growth at 20 ºC as day-1 adults, using an Axioplan microscope and Axiovision software (Zeiss), respectively. At least 30 animals were imaged on at least 2 separate occasions, and results were consistent between experiments.

LipidTOX and Oil-Red-O staining

For LipidTOX staining, animals that had been fed on *E. coli* bacteria containing Nile red or BODIPY-labeled fatty acids as above were washed and fixed as for Oil-Red-O staining (see below). However, instead of dehydrating in 60% isopropanol as in the case for Oil-Red-O, animals were washed free of paraformaldehyde with 1x PBS. 1:1000 of LipidTOX Red neutral lipid stain (for BODIPY-labeled animals) or LipidTOX Green neutral lipid stain (for Nile red-labeled animals) was added directly (LipidTOX DMSO 1000x stocks, Invitrogen). Animals were incubated for 1 hour in the dark with gentle rocking and imaged directly thereafter using a Zeiss Axioimager/Apotome or spinning-disk confocal microscopy.

Oil-Red-O staining was conducted by washing 200-300 day-1 adult animals from plates synchronized by egg-laying with 1x PBS. Worms were washed three times with 1x PBS pH 7.4 and allowed to settle by gravity. To permeabilize the cuticle, worms were resuspended in 120 µl of PBS to which an equal volume of 2x MRWB buffer containing 2% paraformaldehyde (PFA) was added. 2x MRWB buffer: 160 mM KCl, 40 mM NaCl, 14 mM Na_2EGTA, 1 mM spermidine-HCl, 0.4 mM spermine, 30 mM Na-PIPES pH 7.4, 0.2% ß-mercaptoethanol). Samples were gently rocked for 1h at room temperature (allowing animals to rock inside the volume, without spreading the 240µl volume over the whole tube). Animals were allowed to settle by gravity, buffer was aspirated, and worms were washed with 1x PBS to remove PFA. Worms were then resuspended in 60% isopropanol and incubated for 15 minutes at room temperature to dehydrate. Oil-Red-O is prepared as follows: a 0.5g/100mL isopropanol stock solution equilibrated for several days was freshly diluted to 60% with water and rocked for at least 1h, then filtered with 0.45 or 0.22µm-filter. After allowing worms to settle, isopropanol was removed, 1 mL of 60% Oil-Red-O stain was added, and animals were incubated overnight with rocking. Dye was removed after allowing worms to settle, and 200 µL of 1x PBS 0.01% Triton X-100 was added. Animals were mounted and imaged with a Leica colour camera outfitted with DIC optics.

Oil-Red-O quantification

Images were inverted to give a dark background and the blue plane of each RGB image was top-hat filtered to compensate for non-uniform background, then autothresholded to identify regions corresponding to worms. Within these regions, the level of Oil-Red-O was quantified from the original images by determining the excess intensity in the red channel in comparison

to the blue and green channels, with regions with less red than blue or green ignored. These red-excess regions were auto-thresholded to separate background redness from Oil-Red-O stained areas, with mean fatness per image estimated as the total intensity within stained regions normalized by the area of the worm regions.

Fasting analysis

For fasting measurements, adult animals were harvested and washed over a 35 µm mesh (Small Parts) with an excess of S-Basal medium to remove bacteria, and then placed onto NGM plates without (treatment) or with *E. coli* OP50 (fed *ad libitum* control). After 3, 6, or 12 h, worms were re-harvested by washing on a 35 µm mesh to remove progeny, eggs, and bacteria, and frozen on liquid nitrogen for biochemical analysis or immediately stained with Oil-Red-O. Nile red analysis was conducted by washing and transferring animals from plates containing Nile red (25ng/ml) and *E. coli* (on which they were grown from the L1 stage) to plates containing Nile red (25ng/ml) without *E. coli*; control animals were fed continuously on plates containing Nile red.

Quantitative lipid biochemistry

Quantitative lipid biochemistry was conducted as previously described (Soukas et al., 2009). Briefly, 7500 worms at day-1 of adulthood were washed from 3x 10cm plates containing OP50 *E. coli* bacteria and washed over a 20 µm mesh (Small Parts) with an excess of S-Basal medium to remove progeny, eggs, and bacteria. Afterwards, worms were re-harvested and frozen on liquid nitrogen. The total worm pellet was sonicated in 0.25mL PBS, after which we added 50 µL of triglyceride and phospholipid standards dissolved in chloroform:methanol 2:1

(16.7nanomol tritridecanoin, Nu-Chek Prep, and 25 nanomol 1,2-diheptadecanoyl-sn-glycero-3-phosphocholine, Matreya, respectively), and immediately added 2:1 chloroform:methanol up to 1.5 mL to extract lipids. Lipids were extracted for 3h at room temperature. The mixture was spun down (1000xg x 1 min) and the lower organic phase was recovered without debris. The organic phase was washed with 0.3 mL 0.9% NaCl and spun at 1000xg to separate phases. The lower organic phase was transferred again and evaporated under nitrogen. Lipids were resuspended in 100% chloroform to conduct solid phase chromatography using prepacked silica gel columns (Thermo Scientific) followed by GCMS as previously reported (Perez and Van Gilst, 2008; Soukas et al., 2009). For all measurements, at least 2 biological replicates were performed, with data shown as mean ± s.e.m.